BIODIVERSITAS
Volume 24, Number 6, June 2023
Pages: 3394-3406

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240635

Diversity of insect carried-fungi in chili (*Capsicum annuum*) crop at Banyumas District, Central Java Province, Indonesia

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Manuscript received: 19 March 2023. Revision accepted: 22 June 2023.

Abstract. *Suroto A, Mugiastuti E, Tarjoko, Oktaviani E, Bahrudin M. 2023. Diversity of insect carried-fungi in chili* (Capsicum annuum) *crop at Banyumas District, Central Java Province, Indonesia. Biodiversitas 24: 3394-3406.* This study aimed to determine the species of fungal pathogens that have the potency to be carried by various insects in endemic areas of chili (*Capsicum annuum* L.) disease and locations with high populations of chili insect vectors in the Banyumas District. The insect samples were collected from three chili planting locations in Banyumas District (Karanglewas, Sumbang, and Sokaraja Sub-districts) from March to November 2022. The following methods carried out the research: (i) determining the sampling location, (ii) insect sampling and identification, (iii) isolation and purification of fungal pathogen carried by insects, (iv) pathogenicity test, (v) identification of fungal pathogens based on morphology characters. The exploration of 13 villages in 3 (three) sub-districts of Banyumas District found 60 insects collected from healthy and diseased chili plants. Totally, six genera of fungi were isolated and identified, namely *Fusarium*, *Pythium*, *Curvularia*, *Penicillium*, *Geotrichum*, and *Phytophthora*. This is preliminary research on the interaction between plants, microbes, and insects. Understanding these aspects is essential, not only from an ecological perspective but also for improving the genetic quality of crops as well as for integrated pest management.

Keywords: Banyumas District, chili plant, fungi, insect-carried pathogen

INTRODUCTION

Research in respect of insect vectors and their diseases on various plants in Banyumas, which was previously conducted in 2021, is the background for this study. Further analysis will be carried out to ensure the possibility of insects that are intangible as vectors but have the potency to become carriers of plant pathogens. This research must be carried out because insects not acting as vectors can transmit pathogens. Research by Nicholls et al. (2022) showed that flower-visiting insects that assist pollination risk being carriers of pathogens from one plant to another. Another research by Iwebor et al. (2020) explained that insects play a significant role in the spread of pathogens and disease development in sunflowers in the Krasnodar region of the Russian Federation. Furthermore, non-vector insects not only spread pathogens to plants but also can contaminate animal products and feed, releasing harmful toxins to humans and animals (Luo et al. 2018).

Over 700 plant diseases are recognized as vector-borne diseases, adversely affecting crop health and food security worldwide (Jones and Medina 2020). Iwebor et al. (2020) reviewed that there are five forms of the relationship between insects and plant diseases, namely (i) neutral, when insects become mechanical carriers of infective materials, moving from infected plants to healthy plants, (ii) various forms of symbiosis: the insect carries the pathogen within the body, and the pathogen lives within it, (iii) bacteria and viruses pass through the mouthparts and digestive tract of piercing-sucking insects and transmit pathogens through this activity. Then, bacterial binary fission and viral replication occur in the saliva of insects infecting plant fluids, (iv) damage caused by insects contributes to the penetration of microorganisms into the host plant organism, (v) insects transfer saprophytic microflora, forming saprotrophic associations that are not related to the pathological process but continue the process of destruction of plant tissue.

The existence of insect-carried pathogens was also encountered at the initiation test. The first test identified non-vector insect species which carry pathogens. For example, *Liriomyza* spp. (Diptera: Agromyzidae) was identified as a carrier for the pathogen *Colletotrichum* spp. These microorganisms caused anthracnose disease (Rangkuti et al. 2017). This finding was a piece of new information because *Liriomyza* spp. is a leaf-miner insect that lays its eggs in the leaves, and the larvae live in the mesophyll tissue and are not a vector of plant diseases. In the previous study, the insect *Bemisia tabaci* (Hemiptera: Aleyrodidae), a virus vector, was also identified as a pathogenic fungus *Fusarium* spp carrier. There has yet to be any previous research report that *B. tabaci* can be a carrier of *Fusarium* spp. In contrast, *B. tabaci* is known as a virus vector capable of transmitting Tomato Yellow Leaf Curl Virus (TYLCV) (Liu et al. 2013) and is also capable of transmitting more than 400 species of viruses, the majority being Begomoviruses (Geminiviridae) (Ghosh and Ghanim 2021).

However, this research is still an initial screening in Banyumas District, so it still needs further investigation.

Based on the research in the first year, it was found that Karanglewas, Sumbang, and Sokaraja Sub-districts were endemic areas and had high populations of vector insects. This information can be used as the initial data in determining the location for studying insect carriers of plant disease pathogens in the Banyumas District, which will be carried out in this study. Therefore, this research was conducted to discover the pathogens that various insects could carry in disease-endemic areas and locations with high populations of disease vector insects in chili (*C. annuum* L.) plants in the Banyumas District.

MATERIALS AND METHODS

Materials

Materials for this study were healthy chili plants, diseased chili plants, potatoes, bean sprouts, dextrose, agar, chloroform, crystal violet solution, iodine solution, safranin, sodium hypochlorite (NaCl), alcohol, spiritus (denatured alcohol), distilled water, Potato Dextrose Agar (PDA), etc.

The study was conducted in a disease-endemic area with a high population of vector insects on chili plants in the Banyumas District. Samples were collected from 3 (three) chili planting locations in Karanglewas, Sumbang, and Sokaraja Sub-districts from March to November 2022. The research was carried out by following steps, namely: (i) determining the sampling location, (ii) insect sampling and identification, (iii) isolation and purification of insect-pathogenic fungi, (iv) pathogenicity test, (v) identification of fungal pathogens based on morphology characters.

Sampling location

Sampling was conducted in 13 villages from 3 (three) sub-districts that have become endemic locations for chili plant diseases (Figure 1).

Insect sampling and identification

Insects on chili plants were collected by direct capture method using insect nets and aspirators. The collection was carried out 3 (three) times during the vegetative phase and 3 (three) times during the generative phase. Insects were put into a bottle containing cotton (previously dripped with alcohol) for 1 minute. Each species of insect was put into each bottle containing sterile distilled water. Insect sample collection and observation were conducted on ten healthy and diseased plants at each location. The identification of insects at the species level was done using references from Baba and Inoue (1936), Borror and White (1970), Sakagami (1978), and Nakane (1980).

Isolation and purification of pathogenic fungi carried by insects

Isolation from the insect body surface

Each species was dissected into several parts according to their body part. The body part was placed in a sterile petri dish covered with sterile tissue and dripped with sterile distilled water. Incubation was carried out at a temperature of 25-27°C. Fungi that grew on each body part were isolated. Colonies of fungi with different morphology were inoculated on PDA media, added with antibiotics to prevent bacterial growth, then incubated for seven days at 25-28°C.

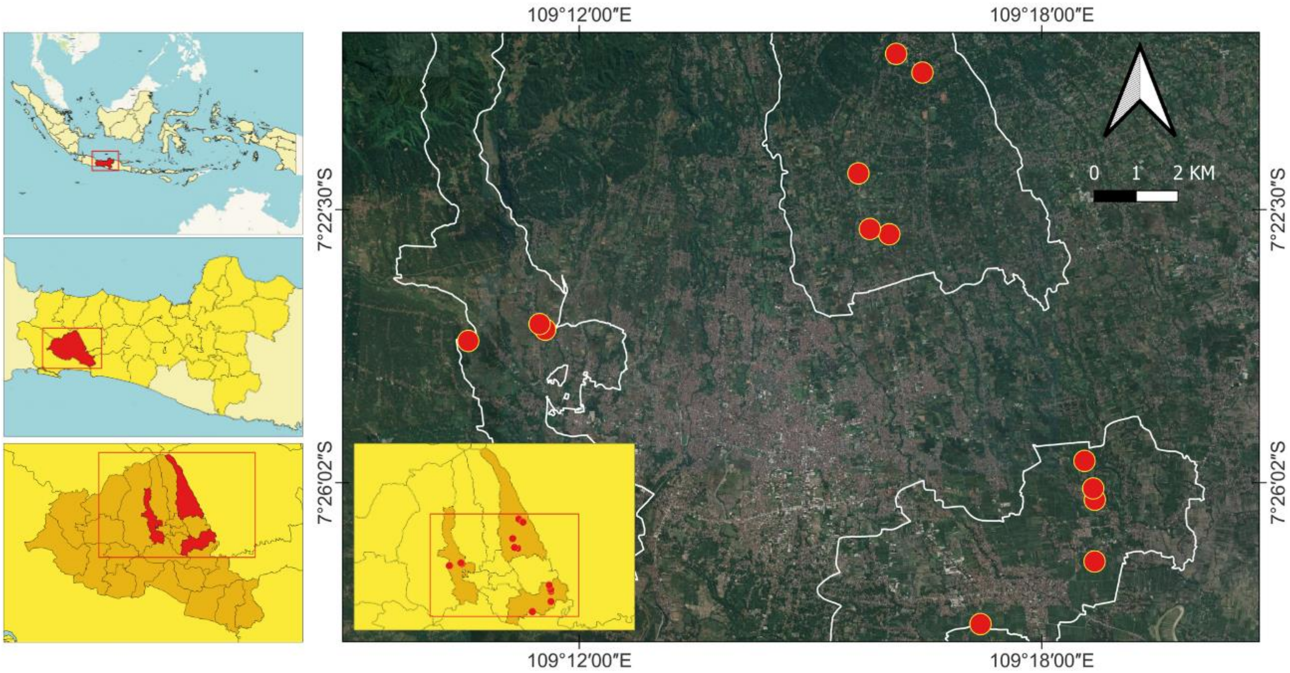

Figure 1. Sampling locations for insect in the Banyumas District, Indonesia

Isolation from inside the body of the insect

The method utilized was a Moist Chamber. The first step was to soak the insects in 70% alcohol for 30 minutes. Then, it was rinsed with distilled water. Body parts such as the head, wings, abdomen, and legs were dissected. These parts were placed in a sterile petri dish covered with sterile tissue and dripped with sterile distilled water. Incubation was carried out for seven days in a humid place with a room temperature of 25-27°C. After incubation, the fungi growing in each media from the different parts were isolated and put into the media for purification. Fungal colonies with different morphologies were inoculated on PDA. Antibiotics were added to prevent bacterial growth, then incubated for seven days at 25-28°C.

Pathogenicity test

Fungal isolates showing hypersensitivity reactions were tested for their pathogenicity on 1-month-old chili seedlings. The fungal inoculation method was carried out using the infectivity titration method by making a wound hole into the plant stem and then inserting a tip containing 10-20 µl of fungal spore suspension into the hole.

Morphological identification of pathogenic fungi

Macroscopic observation of fungi was carried out by observing their shape and color. Microscopic observation was carried out by observing conidiophore branches and the shape of the fungus conidia.

RESULTS AND DISCUSSION

Survey and sampling of pathogen-carrying insects

Samples were collected from healthy and diseased chili plants (Figure 2). Based on the factual observation in the field, the diseased plants encountered were wilting, jaundice, and leaf spot. Overall, 25 species of insects were isolated, which could be categorized into 19 families in 8 orders (Table 1). From these results, 6 species acted as predators, and 19 others acted as pests in chili plants. These insect species belong to the order Coleoptera, Dermaptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, and Thysanoptera.

Identification of insect-carried pathogenic fungi in chili plants

The fungi that were isolated and identified from insects on chili plants in Karanglewas Sub-district are presented in Table 2. There were 15 isolates identified from 3 (three) genera, including *Fusarium*, *Phytophthora*, and *Curvularia*. *Fusarium* sp. was found both from the inside of the insect's body and the outside (Table 2; Figure 3). This fungus was found inside the bodies of *Aphis* sp., *Anastrepha* sp., *Epilachna admirabilis* Crotch, *Delia* sp., and *Nezara viridula* Linnaeus. *Fusarium* sp. was also found on the surface (outside) of the body of the *Oxya* sp., *Musca domestica* Linnaeus, *Anasa tristis* DeGeer, and *Spodoptera litura* Fabricius. In addition, the pathogenic fungus *Phytophthora* sp. was found outside the bodies of *Heteropsylla cubana* Crawford on healthy plants. The pathogenic fungus *Curvularia* sp. was found outside the body of the *Bactrocera* sp.

Table 1. The species of insect found in the sampling locations

Order	Family	Species	Role
Coleoptera	Coccinellidae	*Coccinella transversalis* Fabricius	Predator
		Epilachna admirabilis Crotch	Pest
	Staphylinidae	*Paederus* sp.	Predator
Dermaptera	Forficulidae	*Forficula auricularia* Linnaeus	Predator
Diptera	Tephritidae	*Bactrocera* sp.	Pest
		Euaresta sp.	Pest
		Anastrepha sp.	Pest
	Muscidae	*Musca domestica* Linnaeus	Pest
	Typulidae	*Nephrotoma appendiculata* Pierre	Predator
	Dolichopodidae	*Condylostylus* sp.	Pest
	Anthomyiidae	*Delia sp.*	Predator
Hemiptera	Apididae	*Aphis* sp.	Pest
	Aleyrodidae	*Bemisia tabaci* Gennadius	Pest
	Pentatomidae	*Nezara viridula* Linnaeus	Pest
	Coreidae	*Cletus* sp.	Pest
		Anasa tristis DeGeer	Pest
	Alydidae	*Leptocorisa acuta* Thunberg	Pest
	Psyliidae	*Heteropsylla cubana* Crawford	Pest
Hymenoptera	Apididae	*Apis cerana* Fabricius	Pest
	Formicidae	*Dolichoderus* sp.	Predator
Lepidoptera	Noctuidae	*Spodoptera litura* Fabricius	Pest
Orthoptera	Acrididae	*Gryllus* sp.	Pest
		Oxya sp.	Pest
Thysanoptera	Thripidae	*Thrips* sp.	Pest

Figure 2. Survey and sampling. A. Sample of sampling locations, B. Chili plants infected with jaundice, C. Chili plants infected with wilt disease

The fungi that were isolated and identified from insects on chili plants in Sokaraja Sub-district are presented in Table 3. There were 13 isolates identified from 4 (four) genera, including *Fusarium*, *Pythium*, *Penicillium*, and *Geotrichum* (Table 3; Figure 4).

The *Fusarium* sp. was found both inside the insect's body and outside. *Fusarium* sp. was found in both healthy and diseased plants. This fungus was found outside the bodies of *Aphi*s sp., *Bactrocera* sp., *Anastrepha* sp., *E. admirabilis*, *N. viridula*, and *Apis cerana* Fabricius. In addition, this fungus was also found inside the bodies of *Delia* sp. and *Bactrocera* sp. Another fungus, *Pythium* sp., was isolated from inside the bodies of *Aphis* sp. on diseased plants. *Penicillium* sp. was isolated from the inside of the bodies of *H. cubana* on diseased plants and *Aphis* sp. on healthy plants. In addition, *Penicillium* sp. was also found outside the body of *Dolichoderus* sp. on healthy plants. The fourth fungus, *Geotrichum* sp., was isolated from inside the bodies of *Gryllus* sp. on healthy plants.

The fungi that were isolated and identified from insects on chili plants in Sumbang Sub-district are presented in Table 4. There were 10 isolates identified from 4 genera, including *Fusarium*, *Curvularia*, *Phytophthora*, and *Geotrichum* (Figure 5).

A more varied genus of fungi was obtained at the sampling locations for insect pests and predators in Sumbang Sub-district. *Fusarium* sp. was found both inside and outside the body of insects, both in healthy and diseased plants. From outside the body insect of a healthy plant, *Fusarium* sp. isolated from *Oxya* sp., *Gryllus* sp., *Coccinella transversalis* Fabricius, and *Leptocorisa acuta* Thunberg. *Fusarium* sp. successfully isolated from the *C. transversalis* from a healthy plant's inside body insect. Another fungus which isolated was *Curvularia* sp. This fungus was found outside the body in healthy plants. Other pathogenic fungi, such as *Phytophthora capsici*, have been found outside the body of *A. cerana* from diseased plants. Besides that, *Geotrichum* sp. was found inside the body of *Cletus* sp. on healthy plants.

Table 2. Identification of fungi isolated from the insect in Karanglewas Sub-district

No.	Species	Insect species as a source of fungi	Condition of plants	Source of insect part body
A	*Fusarium* sp. 1	*Aphis* sp.	Healthy	Inside the body
B	*Fusarium* sp. 2	*Anastrepha* sp.	Healthy	Inside the body
C	*Fusarium* sp. 3	*Epilachna admirabilis* Crotch	Healthy	Inside the body
D	*Fusarium* sp. 4	*Delia* sp.	Healthy	Inside the body
E	*Fusarium* sp. 5	*Nezara viridula* Linnaeus	Healthy	Inside the body
F	*Phytophthora* sp.	*Heteropsylla cubana* Crawford	Healthy	Outside the body
G	*Fusarium* sp. 6	*Epilachna admirabilis* Crotch	Healthy	Outside the body
H	*Curvuluria* sp.	*Bactrocera* sp.	Healthy	Outside the body
I	*Fusarium* sp. 7	*Oxya* sp.	Healthy	Outside the body
J	*Fusarium* sp. 7	*Anastrepha* sp.	Healthy	Outside the body
K	*Fusarium* sp. 8	*Musca domestica* Linnaeus	Healthy	Outside the body
L	*Fusarium* sp. 9	*Anasa tristis* DeGeer	Healthy	Outside the body
M	*Fusarium* sp.	*Epilachna admirabilis* Crotch	Healthy	Inside the body
N	*Fusarium* sp.	*Spodoptera litura* Fabricius	Healthy	Outside the body
O	*Fusarium* sp.	*Anasa tristis* DeGeer	Healthy	Outside the body

Table 3. Identification of fungi isolated from the insect in Sokaraja Sub-district

No.	Species	Insect species as a source of fungi	Condition of plants	Source of insect part body
A	*Fusarium* sp. 1	*Aphis* sp.	Healthy	Outside the body
B	*Fusarium* sp. 2	*Bactrocera* sp.	Healthy	Outside the body
C	*Fusarium* sp. 3	*Anastrepha* sp.	Healthy	Outside the body
D	*Fusarium* sp. 4	*Epilachna admirabilis* Crotch	Healthy	Outside the body
E	*Fusarium* sp. 5	*Nezara viridula* Linnaeus	Healthy	Outside the body
F	*Pyhtium* sp	*Aphis* sp.	Diseased	Inside the body
G	*Fusarium* sp.6	*Delia* sp.	Diseased	Inside the body
H	*Penicillium* sp.	*Heteropsylla cubana* Crawford	Diseased	Inside the body
I	*Fusarium* sp. 7	*Bactrocera* sp.	Healthy	Inside the body
J	*Penicillium*	*Aphis* sp.	Healthy	Inside the body
K	*Geotrichum*	*Gryllus* sp.	Healthy	Inside the body
L	*Penicillium* sp.	*Dolichoderus* sp.	Healthy	Outside the body
M	*Fusarium* sp.	*Apis cerana* Fabricius	Healthy	Outside the body

Table 4. Identification of fungi isolated from the insect in Sumbang Sub-district

No.	Species	Insect species as a source of fungi	Condition of plants	Source of insect part body
A	*Fusarium* sp.	*Oxya* sp.	Healthy	Outside the body
B	*Curvularia* sp.	*Aphis* sp.	Healthy	Outside the body
C	*Fusarium* sp.	*Gryllus* sp.	Healthy	Outside the body
D	*Fusarium* sp.	*Coccinella transversalis* Fabricius	Healthy	Outside the body
E	*Fusarium* sp.	*Coccinella transversalis* Fabricius	Healthy	Inside the body
F	*Fusarium* sp.	*Leptocorisa acuta* Thunberg	Healthy	Outside the body
G	*Phytophthora capsici*	*Apis cerana* Fabricius	Diseased	Outside the body
H	*Fusarium* sp.	*Dolichoderus* sp.	Diseased	Outside the body
I	*Fusarium* sp.	*Coccinella transversalis* Fabricius	Healthy	Inside the body
J	*Geotrichum* sp.	*Cletus* sp.	Healthy	Inside the body

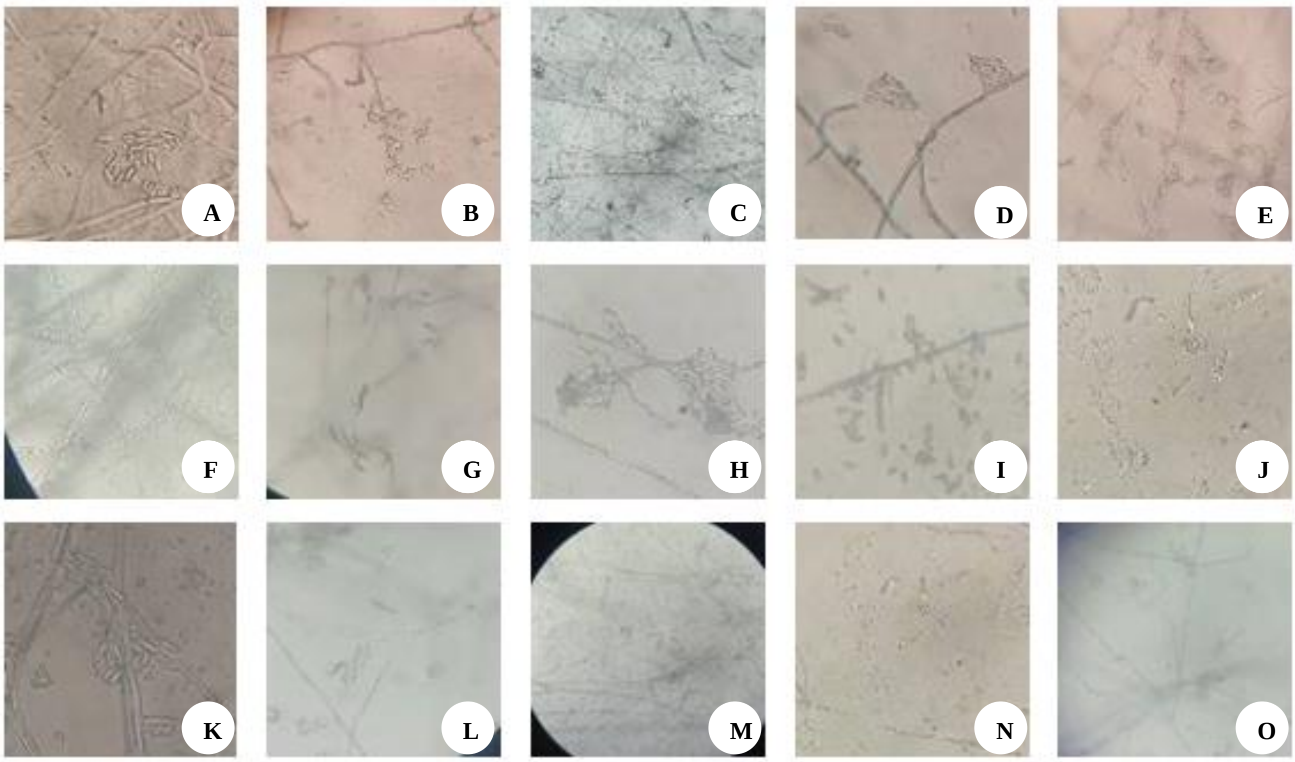

Figure 3. Microscopic fungi photograph isolated from Karanglewas Sub-district (A-O correspond to the fungi identified in Table 2, respectively)

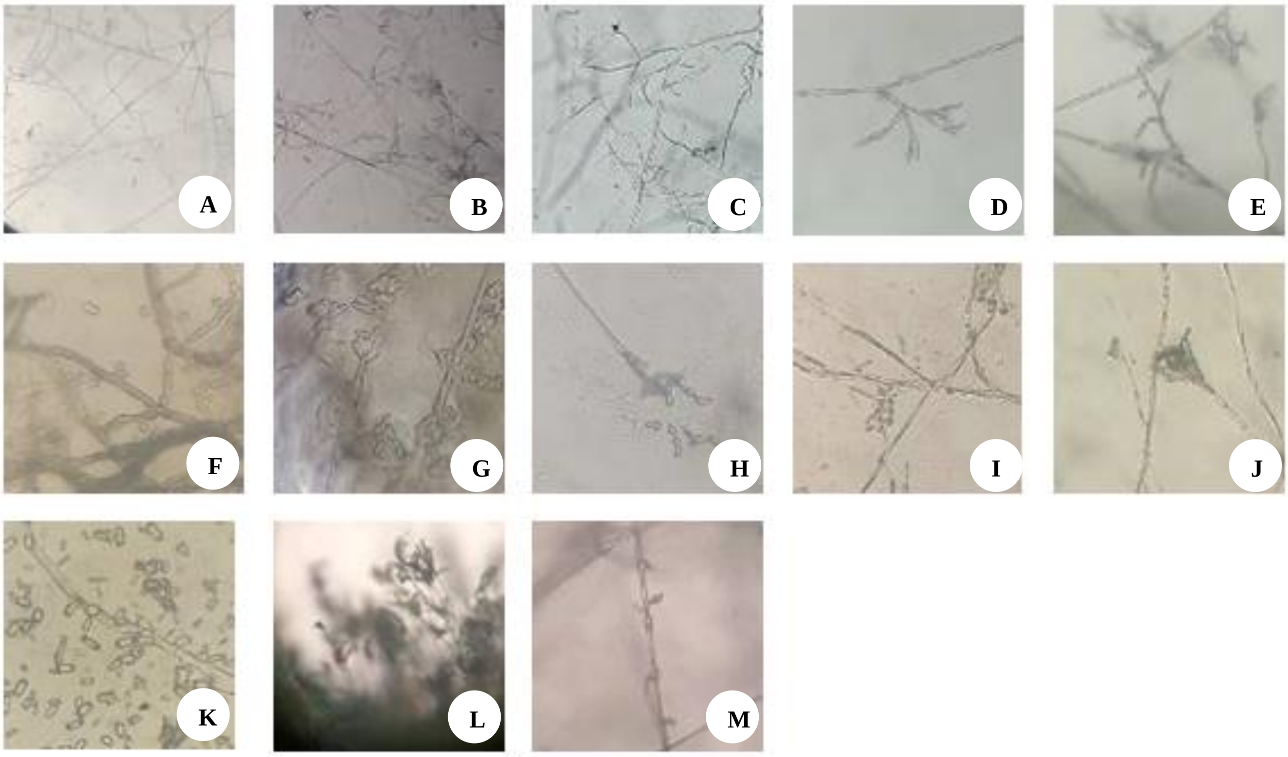

Figure 4. Microscopic fungi photograph isolated from Sokaraja Sub-district (A-M correspond to the fungi identified in Table 3, respectively)

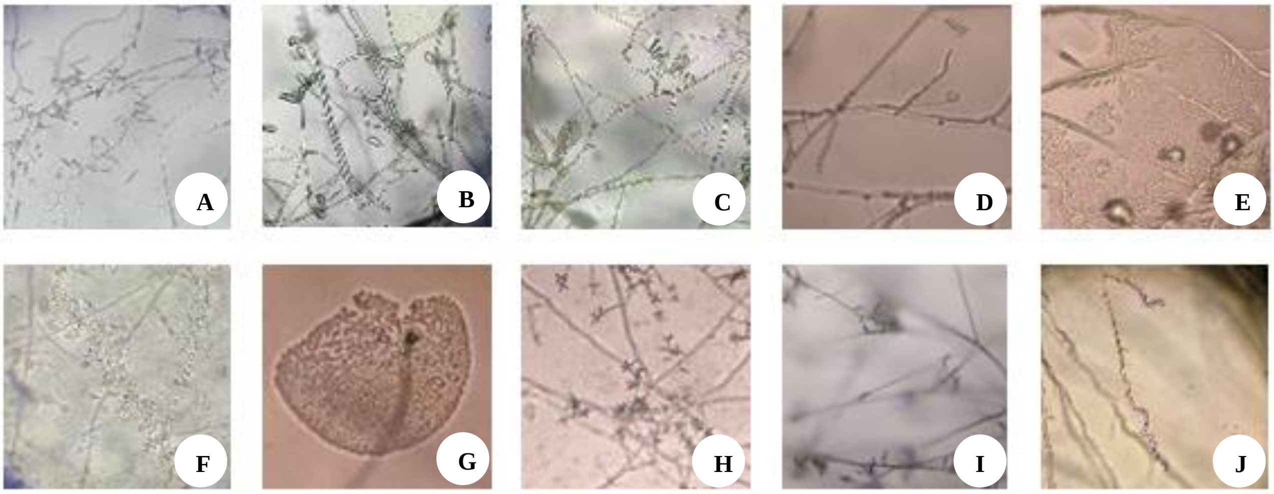

Figure 5. Microscopic fungi photograph isolated from Sumbang Sub-district (A-J correspond to the fungi identified in Table 4, respectively)

Discussion

Diversity of pests and predators in chili plants

According to Agrios (2008), insects can act as pests, reservoirs, or vectors of pathogens because they can cause damage directly by consumption and indirectly by introducing pathogens into tissues injured by bites so that they can spread to all parts of the plant. Wielkopolan et al. (2021) reported that pathogens that insects transmit could induce changes in plant phenotype, palatability, and plant nutrition, to increase the chances of plants being visited by insect vectors and increase the chances of pathogens attacking plants and transmitting pathogens to other plants. The spread of pathogens through insects is one mechanism for disease transmission in a plant.

This research obtained diverse species of insects from the orders Coleoptera, Dermaptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, and Thysanoptera. This result was in line with the reports of Butter (2018) and Chandi (2021), which stated that the orders Hemiptera, Thysanoptera, Diptera, Coleoptera, Hymenoptera, Orthoptera, Lepidoptera, Dermaptera, and Dictyoptera are insect vectors that can carry pathogens from viruses, bacteria, fungi, as well as phytoplasma. Meanwhile, the report of Wielkopolan et al. (2021) showed that 2 (two) insect vectors caused disease in plants from the families Curculionidae and Chrysomelidae, which belong to the order Coleoptera. In a different report, Eigenbrode et al. (2018) also stated that vector insects carrying pathogenic fungi were found in the families Scolytinae (order Coleoptera), Apidae (order Hymenoptera), and Noctuidae (order Lepidoptera). Furthermore, Heck (2018) reported that most insect vectors for plant pathogens come from the order Hemiptera, which generally has a sucking mouth with a stab mode.

This study reported that *C. transversalis*, *E. admirabilis*, and *Paederus* sp. species were found, which belong to the Coleoptera. In the order Dermaptera, *Forficula auricularia* Linnaeus (Family Forficulidae) was found. The species of *Bactrocera* sp., *Euaresta* sp., *Anastrepha* sp., *M. domestica*, *N. appendiculata*, *Condylostylus* sp., and *Delia* sp. were found, which belong to the Diptera. From the Hemiptera, *Aphis* sp., *B. tabaci*, *N. viridula*, *Cletus* sp., *A. tristis*, *L. acuta*, and *H. cubana* were found. From the Hymenoptera, *A. cerana* and *Dolichoderus* sp. were found. From the Lepidoptera, there was *S. litura*, *Gryllus* sp., and *Oxya* sp. were also found, which belong to the Orthoptera. Meanwhile, from Thysanoptera, only one species, *Thrips* sp., was found.

This study contained *C. transversalis*, *E. admirabilis*, and *Paederus* sp. from the Coleoptera. *C. transversalis* and *Paederus* sp. are predator species. In contrast, *E. admirabilis* is a pest. *C. transversalis* is a predator of *Aphis gossypii* (Glover) (Efendi et al. 2018; Sarker et al. 2019), *A. craccivora*, and *A. fabae* (Sarker et al. 2019). No reports have stated that *C. transversalis* was a vector of a particular pathogen. However, studies stated that *Aphis*, a prey of *C. transversalis*, was a vector for Potato Virus Y (Mondal and Gray 2017; Galimberti et al. 2020). Although the nomenclature of the species *Paederus* sp. is unknown, there was a finding about the action of *Paederus fuscipes* as a predator for *A. gossypii* and *Aphis glycines* (Khan et al. 2018). This study can indicate an interaction between predators and animals they prey on and pathogens that these prey animals can carry. Until now, no research has stated the role of *E. admirabilis* as a disease vector insect. However, studies detected the role of the *Epilachna* genus as a virus vector had been carried out by researchers, such as studies on the *Epilachna varivestis*, which is a vector for disease caused by the Bean Pod Mottle Virus (BPMV) virus (Peñaflor et al. 2016; Smith et al. al. 2017; Gedling et al. 2018), Southern Bean Mozaic Virus (SBMV) (Peñaflor et al. 2016), Cowpea Severe Mosaic Comovirus, and Black Gram Mottle Carmovirus (Gedling et al. 2018).

Family Forficulidae (Dermaptera) was also found in this study. There are many species in this family. Sarwar et al. (2020) found the CaMV virus in the digestive tract of the earwig predator *Doru luteipes* Scrudder, a member of the Family Forficulidae. Meanwhile, *F. auricularia* was known to be an insect vector for the fungal pathogen *Sclerotinia fructigena* that caused Brown rot disease in apple plants (Butter 2018).

The species of *Bactrocera* sp., *Euaresta* sp., *Anastrepha* sp., *M. domestica*, *N. appendiculata*, *Condylostylus* sp., and *Delia* sp were found to belong to Diptera. The discovery of species from the Diptera in this study is in line with the research of Arma et al. (2018), which found 4 (four) species of *Bactrocera* sp. in tomato and chili plants. Butter (2018) also reported that a fungal pathogen that caused rots of fleshy fruits was transmitted via *M. domestica*. *N. appendiculata*, an insect pest, has also been reported to be found in wheat plants, which were found in the organs of roots and leaves (Mehrwar and Uniyal 2021). In addition, Cicero et al. (2017) found that various Condylostylus species can prey on Aphids, where Aphids were vectors for Potato Virus Y (Mondal and Gray 2017; Galimberti et al. 2020).

Insects *Aphis* sp., *B. tabaci*, *N. viridula*, *Cletus* sp., *A. tristis*, *L. acuta*, and *H. cubana* were found, which belong to the Hemiptera. The discovery of *Aphis* sp. as a vector for plant viruses is strongly associated with the transmission of fungal pathogens. Meanwhile, the pathogenic fungus *Puccinia punctiformis* is related to Aphids, *Aphis fabae* sp. cirsiiacanthoidis, and *Uroleucon cirsii* (Butter 2018). This study also found *B. tabaci*, a disease vector of Begomovirus that attacks plants of the Cucurbitaceae and Solanaceae families (Li et al. 2021; Subiastuti et al. 2019). In the same order but a different family, Pentatomidae, the insect species *N. viridula* was found. This insect significantly reduced the quality and quantity of soybean (*Glycine max* (L)) cultivation (Azhari et al. 2019; Portilla et al. 2022) and cotton (*Gossypium hirsutum* (L)) (Tillman et al. 2014; Zeilinger et al. 2016; Portilla et al. 2022). This insect was reported as a transmission agent for the bacterial pathogen *Pantoea agglomerans* (Ewing and Fife). It has the potency to become a transmission agent for the pathogenic fungus *Nematospora coryli* (Peglion) in cotton (Tillman et al. 2014; Zeilinger et al. 2016) and *Ralstonia solanacearum* bacteria in bananas (Montong and Salaki 2020). In addition, in the same family, *Cletus* sp. was found. Moreover, different species, *Cletus punctiger*, was known to have a symbiosis with Burkholderia sp. bacteria in the mid-gut digestive tract of this insect (Ishigami et al. 2021). Then, the *L. acuta* species was reported to be a vector for the pathogenic fungus *Helminthosporium oryzae* (Hoesain et al. 2021).

Furthermore, when viewed from the discovery of species in the Order Hymenoptera, the species *A. cerana* itself, which is often called Aphids, was not only an efficient vector for spreading viruses (Yang et al. 2019; Maharani et al. 2020; Guo et al. 2022), but also related to the spread of fungi (Butter 2018). Furthermore, Butter (2018) reported that the fungus *Puccinia punctiformis* was reported to be associated with *Aphis fabae* sp cirsiiacanthoidis and *Uroleucon cirsii*. Then, the *Dolichoderus* sp. reported by Montong and Salaki (2020) carried the pathogenic bacterial species *Ralstonia* *solanacearum* Phylotype IV, the cause of Banana Blood Disease (BBD).

There has been no report that *S. litura*, which belongs to the Lepidoptera, was a disease vector. However, in the same genus, the insect species *Spodoptera ornithogalli* was known to be able to attract the endophytic fungi *Claviceps pasoli* and *Fusarium heterosporum* (Butter 2018). Meanwhile, in the Orthoptera, Aleknavičius et al. (2022) reported the presence of bacterial microbiota in cricket species, *Acheta domesticus* and *Gryllus assimilis*, using metagenome analysis techniques (whole genome sequencing), apart from *Gryllus* sp. *Oxya* sp. (Orthoptera) was also found in this study.

In the Thysanoptera, the species *Thrips* sp. which belongs to the Family of Thripidae was reported as a vector insect pest of the virus that caused tospovirus disease (Marianah 2020). On the other hand, Butter (2018) reported the presence of fungi isolated from thrips, both of which were pathogenic such as *Fusarium oxysporum*, *Phoma medicaginis*, *Ulocladium* spp., *Penicillium* spp., *Alternaria* spp., as well as those that were non-pathogenic.

Identification of pathogenic fungi carried by pests and predators in chili plants

Fusarium sp.

Fusarium sp. is the fungus species classified in the Division of Ascomycota, class Sordariomycetes, order Hypocreales, and family Nectriaceae (Aoki et al. 2014). *Fusarium* sp. is often found in the soil (Aoki et al. 2014; Dusengemungu 2021). This species is commonly known as a plant disease pathogen; some species are entomopathogenic (Dusengemungu 2021). In addition, this fungus species can be utilized as a biofertilizer.

Observations both macroscopically and microscopically correspond to the characteristics of *Fusarium* sp. The characters were mycelium proliferates in cultures resembling cotton, and often the mycelium is slightly pink, purple, or yellow in the media; variation in conidiophores, having a cylindrical shape, simple or strong, short, irregularly branched or connected to a phyalida, or collected in sporodochia; hyaline conidia, two forms often attached to the tips; the macroconidia consist of several cells, have a slightly curved shape or are bent at the ends; single-cell microconidia have an ovoid shape, single or in chains; some conidia sometimes consist of 2 or 3 cells, rectangular or slightly curved (Hidayah et al. 2021). According to Funder (1953), conidia develop directly on the side of the hyphae without any detectable conidiophores called sessile and lateral (on the sides). Conidia can be produced laterally on the short, clustered hyphae branch, such as grapevines.

The epidemiology of *Fusarium* wilt disease caused by *Fusarium* in chili starts from the ability of the fungus to survive freely in the soil because it can colonize the roots of weeds and produce resistant spores. The fungus usually penetrates the host through a wound in the root. Then, it multiplies and colonizes the vascular system. Infection occurs at any time during the cycle of plant life. The level of disease will be more acute when air and soil temperatures match those of the fungus, at around 25-32ºC (Sikora 2004).

Wilt disease symptoms include drooping and yellowing of the lower leaves, followed by the whole plant. The leaves of the affected tissue are still attached, and the vascular system changes color, especially the lower stems and roots (Jain et al. 2019). Pathogens live freely in the soil and are dispersed by irrigation water. Pathogens are very susceptible to alterations in temperature and soil moisture. Cruz et al. (2019) reported that *F. oxysporum* showed in vitro radial growth the best after incubation at 25ºC and pH 6. Other fungi, including *P. capsici*, can cause wilt disease on chili plants (Guigón-López et al. 2019; Moreira-Morrillo et al. 2022; Santos et al. 2023), and Fusarium wilt disease is also caused by the fungus *F. oxysporum* f.s (Din et al. 2020; Rahman et al. 2021). The chili attacked by the two species of pathogens showed almost similar symptoms. The plants wilt due to damage to the vessel of water and nutrient transport channels, both on the roots and the stems.

In diseases caused by fungi, humidity affects the longevity of fungal spores, especially the germination of spores requiring a layer of water covering plant tissue to germinate. Most pathogenic fungi depend on the presence of water on the host or high relative humidity in the air only during the germination of their spores and are entirely independent of these conditions. Spores must germinate, and at that status, they need a suitable temperature and water in the form of rain, dew, or a layer of water on the plant surface or at least requires a high relative humidity (Sephton-Clark and Voelz 2018).

Penicillium sp.

Penicillium sp. is classified into the division Ascomycota, class Eurotiomycetes, order Eurotiales, and family Trichocomaceae (Houbraken et al. 2020; Tsang et al. 2018). Cosmopolitan fungi, with a wide distribution throughout the world, are often found growing on the upper surface of the soil, playing a role in the decomposition and composting of organic material (Naranjo-Ortiz and Gabaldón 2019). *Penicillium* sp. has septate hyphae and forms conidia (Hidayah et al. 2021). Conidia have branches called phialides, which look like a bubble (Mirsam et al. 2022). Conidial stalks are called conidiophores. The fruit body usually has a broom-like shape followed by a chain-like sterigma and conidia (Hidayah et al. 2021).

Pythium sp.

Pythium sp. is classified into the division Heterokontophyta, class Oomycetes, order Pythiales, and family Pythiaceae (Ho 2018). *Pythium* is fungi that cause soil-borne disease in the seeds of various plants and damping off (Khrieba 2020). The genus *Pythium* has a coarse mycelium up to 7µm wide (Ho 2018), sporangium round and oblong. In indirect germination, the sporangium protoplasts come out and form vesicles, then the vesicles undergo differentiation to form flagellated zoospores outside the sporangium. Sporangium generally has an irregular shape (pre-sporangium). Oospores have a smooth, thick shape (17-19 µm in diameter) resulting from fertilization between the antheridium and the oogonium. In culture media, this fungus forms spherical chlamydospores (21-39 µm in size) (Sujadmiko 2015).

Phytophthora sp.

Phytophthora sp. infection on the roots or tip of the stem causes wilting followed by rapid death, with attack intensity ranging from 55.66%-61.20% (Bande et al. 2014). The shape of sporangium of *Phytophthora* sp has a round shape (globose, ellipsoid, and ovoid), a pear-like shape (obpyriform and obturbinate), lemon (limoniform), and an irregular shape (distorted). The length (l) of the sporangium ranged from 15.1-76.2 µm, the width (w) was 9.8-44.8 µm, and the ratio of l/w sporangium was 1.12-2.27 (Table 1). All isolates have a clear papilla at the end of the sporangium. Based on the criteria Erwin and Ribeiro (1996) presented, hypha branching showed a similar umbrella type (sympodial umbel). Some sporangiophores come from one place, and a sporangium forms at the ends.

Phytophthora sp. is a pathogenic fungus that macroscopically has white colonies and smooth, flat surfaces like cotton. The direction of mycelium growth is sideways and upwards. Colonies regularly form a circle. Microscopically, *Phytophthora* sp. has brown egg-shaped spores with a length of 8.75-12.5µm. The Conidia size of *Phytophthora* sp. reported by Ivayani's research (2018) has an average diameter of 14.2-19 µm × 14.4-22.2 µm. The mycelium produces branched sporangiophores, which produce lemon-shaped sporangia at the ends of the sporangiophores. The swelling of the mycelium into sporangiophores is the main characteristic of *Phytophthora* sp. (Agrios 1997).

Curvularia sp.

Curvularia sp. is a seed-borne pathogenic fungus. Colonies are grey-black, have a smooth, thin surface like cotton, the growth direction is sideways and upward, the colony base is black, forms a dense ring zone, and the colony shape is regular in a circle. Based on its microscopic characteristics, the hyphae of *Curvularia* sp. are hyaline and insulated. Conidia of *Curvularia* sp. are shaped like a crooked rod that is hyaline, black, and has three partitions. The conidial size of *Curvularia* sp. has a length between 18.75-26.25 µm and a width between 11.25-15 µm. Based on these macroscopic and microscopic characteristics, it is predicted that the fungus found is *Curvularia* sp.

The result of the research is supported by Sobianti et al.'s (2020), who described the macroscopic and microscopic characteristics of *Curvularia* sp. The colonies lack color, with a smooth surface like cotton. Meanwhile, this fungus's microscopic feature is its pale to blackish conidia with a slightly curved shape. The conidia of this fungus have three partitions, with the third cell being larger and darker than the other cells. Amteme and Tefa (2018) explained in their research that the conidia of *Curvularia* sp. have a brown color and size between 16-26 µm × 8-12 µm.

Geotrichum sp.

This fungus has white hyphae, and the colony base is white. The shape of the edges of the colony is round, the colony's surface is smooth and flat, and the hyphae are thin. Hyphae developed quickly within 1-2 days after isolation on the media, and the cup colonies filled the petri dish after 3-4 days. The colony's color changed in the 5-7 days from white to brownish grey or dark grey. The microscopic characteristics of the fungus *Geotrichum* sp. are segmented and brown, which has hyphae and a cylindrical or tube shape and a stalk-like shape. The features of the fungus *Geotrichum* sp. in this study are in line with the results of Dugan's study (2006) that the fungus of the genus *Geotrichum* has white, septate mycelium, arthrospores conidia, clear conidia originating from hyphae segmentation, one cell, has a short cylindrical shape.

Researchers have conducted studies on the diversity of insect-borne fungi at various locations. Melsilawati et al. (2012) found the genera *Acremonium, Aspergillus, Debaryomyces, Hanseniaspora, Fusarium, Penicillium,* and *Geotrichum* in the intestines of the house fly (*M. domestica*). Some of the fungi in this study were also found in Sales et al. (2002) and Banjo et al. (2005) in Iran and Nigeria. *Fusarium oxysporum, Aspergillus tamari,* and *Penicillum axalicum* were isolated from the body surface of *M. domestica,* while *Alternaria* sp., *F. oxysporum,* and *Cladosporium* sp. are found in the gut of *M. domestica* (Sales et al. 2002; Banjo et al. 2005). These fungi can be parasitic or saprophytic.

In addition to these studies, Butter (2018) reported the presence of *F. oxysporum* and *Penicillium* spp, which are pathogenic, isolated from thrips and other pathogenic fungi such as *Ulocladium* spp., *P. medicaginis*, and *Alternaria* spp. Thrips eat the spore of this fungi as a food source. Another way of spreading the organism is through the body being contaminated with spores so that the insect can mechanically spread the fungus as a pathogen carrier. In the same report, the insect *S. ornithogalli* was reported to be able to attract the endophytic fungi *C. pasoli* and *F. heterosporum*, so there was an interaction between insects and mold. According to Batubara (2002), the relationship between fungi and the digestive system of insects was that microorganisms were assimilating food, changing or destroying substances contained in digestion, and can produce and release enzymes for a particular activity.

Factors influenced the existence of pathogenic fungi from inside and outside the body of insects

Various factors can cause fungal microorganisms to be isolated in the bodies of insects. Chapman (2013), Engel and Moran (2013) reported that the main structure of the insect digestive system is similar in all orders, which consists of 3 (three) main parts, namely the foregut, midgut (ventriculus), and hindgut. The structure of each order showed variations in modifications of certain parts because it is related to adaptation to different feeding habits and habitat niches (Chapman 2013). These structural specializations were known to have evolved to provide habitats for certain microorganisms (Engel and Moran 2013). The physicochemical conditions influenced the colonization of microorganisms inhabiting the insect digestive system in the lumen of the different digestive system compartments, especially pH and oxygen availability (Engel and Moran 2013; Gupta and Nair 2020), the stage of development of the insect (Engel and Moran 2013; Yun et al. 2014; Zhang et al. 2018), the ability of bacteria cell to divide (Engel and Moran 2013), availability of nutritional sources or insect feed, environmental factors, and host insect phylogeny (Yun et al. 2014; Yuning et al. 2022). In a more specific context, Zhang et al. (2018) reported that the bacterial and fungal communities in the digestive system of the insect *A. mali* (Coleoptera: Buprestidae) were influenced by the stages of insect development and the host insect feed.

In this study, fungi were also isolated outside the insect's body. According to Sanjaya et al. (2018), apart from the digestive tract, the insect cuticle is a part that can become a reservoir for microorganisms. The surface of the insect's body, which contains organic materials such as chitin, is the first barrier for microorganisms that will infect the inside of the insect's body. In addition to chitin, according to Ortis-Urquiza and Keyhani (2013), the composition of the insect's body surface consists of layers of wax and cement, including various types of hydrocarbon compounds (alkanes, alkenes, and various other methyl-branched derivative compounds), fatty acids and esters, alcohols, ketones, and aldehydes, as well as minor compound components in the form of triacylglycerols, epoxides, ethers, and tanned proteins that affect and direct essential aspects of environmental interactions and insect behavior. Furthermore, Morris et al. (2013) stated that microorganisms could occupy around 10% of insect biomass.

Differences in responses of chili plants to exposure to fungi carried by pests and predators

This study found pathogenic fungi in both diseased and healthy plants. The interaction between pathogens, plants, and insect vectors that carry diseases is complex. In chili plants that are still healthy, while these plants interact with pathogenic fungi carried by pests and predators, this relationship does not cause chili plants to become diseased, or it can be said to have a neutral relationship. This can also be caused by the plant's immune system against exposure to pests (or predators) and pathogens. According to Noman et al. (2020), interactions between plants, microorganisms, and insects could affect plant immunity, insect behavior, and disease development processes (pathogenesis). The tripartite relationship that occurs has yet to reach the threshold for the appearance of diseased plant symptoms at the tissue and organ level.

According to Shittu et al. (2019) and Paludan et al. (2021), plants have mechanisms of resistance regulation throughout the growth and reproduction phases with significant energy requirements. Plants not only distinguish herbivorous insects from the mechanical damage (wounds) they cause to the tissues but also from the secretions (saliva and vomit of insects) that are deposited on the injured tissues (Ray et al. 2015). It is these molecules deposited by insects that induce plant responses, known as elicitors or Herbivore-Associated Molecular Patterns (HAMPs), and are analogous to Pathogen-Associated Molecular Patterns (PAMPs) or Microbe-Associated Molecular Patterns (MAMPs) (Schmelz 2015). The elicitors released by these insects have molecular effectors, which will bind to receptors on the surface of plant cells, which determines the success of herbivores in interfering with the stability of the plant immune system (Fürstenberg-Hägg et al. 2013; Schuman and Baldwin 2016; Basu et al. 2018; Gully 2019; Malik et al. 2020). According to Schmeltz (2015), the sequence of responses of plant resistance to herbivorous insects was interactions with damage, deposits of components of oral secretions or glandular secretions, interactions of ligands (from herbivores)-plant receptors, ion flow, signaling of protein kinases, interactions with phytohormones, activation of transcription factors, changes in the mechanism of translation, ending with the biosynthesis of various resistance compounds.

In the tripartite relationship that occurs, the response given by plants to insect attacks and pathogenic microorganisms can result in changes in aspects of plant physiology and molecular biochemistry, such as gene expression and reprogramming of transcription mechanisms (Franco et al. 2017). Plants receive signals in molecular patterns associated with herbivory or insect bite injury through receptors activating early signaling components such as Ca^{2+}, Reactive Oxygen Species (ROS), and Mitogen-Activated Protein (MAP) kinases (Erb and Reymond 2019). Systemic and local regulation of toxins, pest and pathogen resistance proteins, physical inhibitors, and various other stresses are thought to occur in chili plants exposed to pests and pathogens, resulting in chili plants not showing symptoms of diseased plants. Systemic and local regulation can result in a series of molecular and cellular changes to produce metabolite compounds that play a role in plant resistance. These compounds can be volatile or non-volatile, as well as overall changes in the profile of compounds in plants and compounds for visual cues for insects (Bouwmeester et al. 2019; Anton and Cortesero 2022). Metabolic changes can be positive or negative, affecting the resistance and behavior of insects, along with the spread of pathogen infection (Jeandet et al. 2013; Panini et al. 2016; Ebert 2019; Gorshkov and Tsers 2021; Zhao et al. 2023). Changes due to systemic and local regulation allow for plant resistance responses at the molecular and cellular levels and tissues. Still, they do not result in tissue and organ damage, a symptom of diseased plants. Understanding the love and hate-relationship between plants, microbes, and insects is important, not only from an ecological perspective but also for improving the crops' genetic quality and integrated pest control.

ACKNOWLEDGEMENTS

The author would like to thank the Institute for Research and Community Service, Universitas Jenderal Soedirman, Indonesia, for the Competency Improvement Research grant in 2022, with a contract number T/710/UN23.18/PT.01.03/2022.

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